DATA EVALUATION RECORD

DICAMBA

Study Type: OCSPP Non-Guideline; Gene Mutation Assay in Transgenic Mice

EPA Contract No. EP-W-16-018
Task Assignment No. 34-3-001
(MRIDs 51129105, 51129106, 51129107, 51129108, and 51129109)

Prepared for
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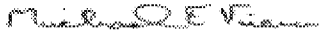
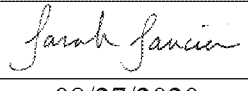
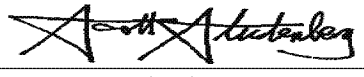
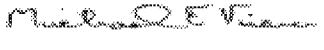
Primary Reviewer: Michael E. Viana, Ph.D.	Signature: Date:	 08/21/2020
Secondary Reviewer: Sarah E. Saucier, Ph.D.	Signature: Date:	 08/27/2020
Quality Assurance: Scott D. Studenberg, Ph.D., DABT	Signature: Date:	 08/28/2020
Project Manager: Michael E. Viana, Ph.D.	Signature: Date:	 08/31/2020

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. Contractor's role did not include establishing Agency policy.

EPA Reviewer: Sarah Dobreniecki
Risk Assessment Branch VII, HED (7509P)

Signature: Sarah Dobreniecki
Date: 9/16/2020
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Gene Mutation Assay in Transgenic Mice; OCSPP Non-Guideline; OECD 488.

PC CODE: 029801 **DP BARCODE:** D458715

TXR #: 0058082

TEST MATERIAL (PURITY): Dicamba (89.8% a.i.)

SYNONYMS: BAS 183 H; SAN837 technical; 3,6-dichloro-2-methoxybenzoic acid

CITATION: Ueda, M. (2020) Dicamba techn. (BAS 183 H; SAN837 techn.): transgenic mice (Muta™Mouse) gene mutation assay. BioSafety Research Center, Inc., Shizuoka, Japan. Laboratory Study ID: 886458, March 13, 2020. MRID 51129105. Unpublished.

Ueda, M. (2020) Dicamba techn. (BAS 183 H; SAN837 techn.): dose range-finding study for transgenic mice (Muta™Mouse) gene mutation assay. BioSafety Research Center, Inc., Shizuoka, Japan. Laboratory Study ID: 886460, March 10, 2020. MRID 51129106. Unpublished.

Blum, M. (2020) Validation of an analytical method for the analysis of BAS 183 H (dicamba techn.) in powdered diet, CRF-1 (Oriental Yeast) using HPLC-UV (Control procedure 97/0267_03). BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany. Laboratory Study ID: 887672, February 3, 2020. MRID 51129107. Unpublished.

Bangert, L. (2020) BAS 183 H (Dicamba techn.): homogeneity and concentration control analysis in powdered diet CRF-1 (Oriental Yeast). BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany. Laboratory Study ID: 887680, January 30, 2020. MRID 51129108. Unpublished.

Wagner, I. (2020) BAS 183 H (Dicamba techn.): stability analysis in powdered diet CRF-1 (Oriental Yeast). BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany. Laboratory Study ID: 887679, February 11, 2020. MRID 51129109. Unpublished.

SPONSORS: Syngenta, Ltd., Jealott's Hill International Research Centre, Bracknell, Berkshire, UK
BASF SE, Ludwigshafen, Germany

EXECUTIVE SUMMARY: In a non-guideline, gene mutation assay (MRID 51129105), groups of seven male Muta™Mouse (CD_2-LacZ80/HazfBR) mice/dose level were administered dicamba (89.8% a.i., batch # P.MG2726410) in the diet at dose levels of 0, 1200, 3000, or 7000 ppm (equivalent to 0, 176.4, 431.1, and 924.9 mg/kg/day) for 28 consecutive days. After a three-day period for mutations to become fixed in the genomic DNA, the mice were euthanized on Day 31. A positive control group of seven male mice were administered *N*-ethyl-*N*-nitrosourea (ENU) in 1/15 mol/L sodium phosphate buffer (pH 6.0) by i.p. injection (dose volume 10 mL/kg) at a dose level of 100 mg/kg/day; two doses were given approximately 24 hours apart on Days 3 and 4. After ten days, these mice were euthanized on Day 14. The duodenum was examined for genomic DNA mutations induced by test substance exposure.

There were no effects of treatment on clinical signs of toxicity, absolute or relative (to body) duodenum weights, or necropsy or microscopic findings. There were no changes in absolute or relative (to body) duodenum weights in the ENU-treated group.

In the 7000 ppm group, there were decreases in body weight on Days 15 (↓6%) and body weight gain during Days 1-31 (−0.1 g treated vs 0.7 g control). Additionally, at 7000 ppm, food consumption was decreased by 22% during Days 1-3. In the positive control group, there was a decrease in body weight during Days 1-14 (↓8%).

Administration of dicamba in the diet did not increase mutant frequency. All mean values fell within the 95% confidence range (14.4×10^{-6} to 86.9×10^{-6}) calculated from the historical control data. The positive control (ENU) produced a marked 10.6-fold increase in the mutant frequency.

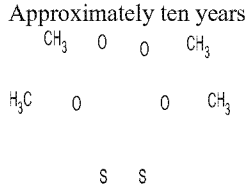
This study is classified as **acceptable / non-guideline**.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound Dicamba (BAS 183 H; SAN837 techn.)

Description:	White solid
Lot #:	P.MG2726410
Purity:	89.8% a.i.
CAS # of TGAI:	1918-00-9
Expiration/storage:	Approximately ten years stored at room temperature
Structure:	

2. Vehicle: Diet.

3. Test animals

Species:	Mouse (male only)
Strain:	CD_2-LacZ80/HazfBR (Muta™Mouse) transgenic mouse
Age / weight at Day 1:	11 weeks / 25.5-29.7 g
Source:	Japan Laboratory Animals, Inc.
Housing:	Individually in metallic mesh cages with an aluminum tray and bedding.
Diet:	Powdered CRF-1 diet sterilized by irradiation (Oriental Yeast), *ad libitum*.
Water:	Tap water, *ad libitum*.
Environmental conditions	
Temperature:	22.8-23.1°C
Humidity:	47-57%
Air changes:	≥12 / hour
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	Approximately 8 weeks

B. STUDY DESIGN

1. Introduction: It was stated that the aim of the present study was to investigate if dicamba could induce gene mutations (reporter gene: *lacZ*) in transgenic mice (Muta™Mouse) following dietary administration for 28 consecutive days by using the transgenic rodent (TGR) mutation assay. The TGR mutation assay was conducted according to the OECD TG-488 test guideline. The assay is used for the detection of *in vivo* mutations induced by the test substance by analyzing target organs. Genomic DNA is extracted from target organs of mice administered the test substance. The target gene is recovered from the genomic DNA by incorporation of the transgene into a λ bacteriophage or plasmid shuttle vector. Mutations are *in vivo* mutations that arise in the mouse and are detected by positive selection methods. The TGR mutation assay with *lacZ* bacteriophage mouse (Muta™Mouse) was considered appropriate for an evaluation of potential mutations because it has sufficient historical control data.

2. In-life dates: Start: October 29, 2019 End: November 28, 2019

3. **Animal assignment:** The mice were randomly assigned to the groups presented in Table 1 based on body weight on Day 1. It was stated that the weight range was not to exceed ±20% of the mean weight.

TABLE 1: Study design [a]

Test group	Dietary concentration (ppm)	Compound intake (mg/kg/day)	# males [c]
Control	0	0	7
Low	1200	176.4	7
Mid	3000	431.1	7
High	7000	924.9	7
Positive control [b]	---	100	7

a Data were obtained from pages 34 and 43 of MRID 51129105.
b Positive control = 100 mg/kg/day *N*-ethyl-*N*-nitrosourea (ENU), administered by two i.p. injections approximately 24 hours apart.
c Seven mice/group were treated but only six mice/group were evaluated.
--- Not applicable

The first day of administration was designated as Day 1. The dietary formulations were administered during Days 1-28. The positive control mice were fed basal diet and were administered ENU by i.p. injection on Days 3 and 4 approximately 24 hours apart.

4. **Dose selection rationale:** The doses used in the present study were selected based on a dose range-finding study (MRID 51129406). A summary of this study is presented as Appendix 1 at the end of this DER.

5. **Diet preparation and analysis:** The dietary formulations were prepared by weighing an appropriate amount of the test substance (corrected for purity) and mixing it with basal diet using a mortar and pestle to form a premix. The premix was sieved (600 µM), transferred to a mixer, and combined with additional basal diet to yield the desired dietary concentration. The formulations were stored in sealed containers at room temperature and used within eight days.

A validation study (MRID 51129107) for an analytical method to detect dicamba in the basal diet is presented as Appendix 2 at the end of this DER.

Homogeneity and concentration analyses (MRID 51129108[1]) and stability analyses (MRID 51129109[2]) were presented in separate, concurrently-reviewed documents and are summarized here. Six samples (three samples from the top and bottom strata each) were randomly collected from each test substance formulation (one sample of the control/basal diet) and analyzed for homogeneity and concentration. Stability analyses were performed after completion of the definitive study (December 2019) on a 1000 ppm dietary

[1] Bangert, L. (2020) BAS 183 H (Dicamba techn.): homogeneity and concentration control analysis in powdered diet CRF-1 (Oriental Yeast). BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany. Laboratory Study ID: 887680, January 30, 2020. MRID 51129108. Unpublished.

[2] Wagner, I. (2020) BAS 183 H (Dicamba techn.): stability analysis in powdered diet CRF-1 (Oriental Yeast). BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany. Laboratory Study ID: 887679, February 11, 2020. MRID 51129109. Unpublished.

formulation; five samples were randomly collected following 0, 1, 3, 8, and 9 days storage at ambient temperature.

Results

Homogeneity (%RSD): 2.7-3.6%

Stability (% of time 0): 96.6% after 9 days storage at ambient temperature

Concentration (% nominal): 104.8-123.6%

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable. The variance between nominal and actual dosage was greater than normally accepted; however, because this is a special study and the high dose was intended to approximate a limit dose of 1000 mg/kg/day, the Reviewers do not consider this to be a major deficiency.

6. **Positive control preparation:** The positive control (ENU; *N*-ethyl-*N*-nitrosourea) was prepared by weighing the required amount and diluting it with 1/15 mol/L sodium phosphate buffer (pH 6.0) to yield a 10 mg/mL solution just prior to use. Formulation analyses were not reported.

7. **Statistics:** The following statistical analyses were performed. All statistical tests were two-tailed and significance was denoted at $p \leq 0.05$.

Parameter	Analyses
Body weights, body weight gains, absolute and relative (to body) organ weights, and food consumption	Group means were analyzed with Bartlett's test for homogeneity of variance. If Bartlett's test was not significant ($p>0.05$), Dunnett's multiple comparison test was used to compare the treatment groups to the control. If Bartlett's test was significant ($p \leq 0.05$), Steel's test was performed to compare the treatment groups to the control.
Mutation frequency	Group means for the treatment groups and the control were analyzed as above. Group means for the control and positive control groups were analyzed with an F test for homogeneity of variance. If the F test was not significant ($p>0.05$), Student's t-test was performed. If the F test was significant ($p \leq 0.05$), an Aspen-Welch t-test was performed.

The Reviewers consider the statistical analyses used appropriate.

C. METHODS

1. **Observations:** The mice were observed for mortality and morbidity twice daily on weekdays during dosing and once daily on weekends and between dosing and euthanasia.

2. **Body weights and body weight gains:** The control and treated mice were weighed on Days 1, 3, 8, 15, 22, 29, and 31 (just prior to euthanasia). The positive control (ENU) mice were weighed on Days 1 and 14 (just prior to euthanasia).

3. **Food consumption and compound intake:** Food consumption (g/mouse/day) of the control and treated mice was determined by weighing the feeder on Day 1, 3, 8, 15, 22, and 29. It was stated that compound intake (mg/kg/day) was calculated but the method was not reported. (The Reviewers assume the calculations used the food consumption and body weight data and the nominal dietary concentrations.)

4. **Sacrifice and pathology:** The control and treated mice were euthanized by blood sampling/exsanguination under isoflurane anesthesia; the positive control mice were euthanized by exsanguination under isoflurane anesthesia. All mice were given a complete necropsy; the following tissues (X) were collected and the (XX) tissues were weighed.

XX	Duodenum	X	Kidney	X	Lymph node (mesenteric)
X	Liver	X	Heart	X	Testis
X	Stomach	X	Bladder	X	Vas deferens/cauda epididymis

Organs and tissues were processed as follows. Following processing, all tissues were stored in an ultralow temperature freezer.

a. **Duodenum:** The duodenum was examined macroscopically, weighed, and an approximately 6-cm section from the pylorus of the stomach was incised and the contents removed by flushing with isotonic saline. The duodenum was then cut into three segments approximately 2 cm long each. The section nearest the pylorus was fixed in 10% neutral-buffered formalin and the other two sections were frozen in liquid nitrogen. The fixed duodenum was processed routinely, stained with hematoxylin and eosin, and examined microscopically.

b. **Liver:** Two samples were obtained from the left lateral lobe and frozen separately in liquid nitrogen. The remaining lobes were placed in a storage bag, compressed, and frozen with a flat-bottomed metal container filled with liquid nitrogen. The remaining left lateral lobe was fixed in 10% neutral-buffered formalin.

c. **Stomach:** The stomach was incised and the contents removed by flushing with isotonic saline. A portion of the stomach (including forestomach and glandular stomach) was trimmed to approximately 4 × 10 mm and fixed in 10% neutral-buffered formalin. The remaining part was separated into forestomach and glandular stomach (two pieces), placed into separate storage bags, and frozen in liquid nitrogen.

d. **Kidney:** The capsule of the left kidney was removed, and two 1-2 mm thick pieces of the left kidney were sliced off and frozen separately in liquid nitrogen. The capsule of the right kidney was removed and the right kidney was fixed in 10% neutral-buffered formalin. Remaining parts were compressed in a storage bag and frozen with a flat-bottomed metal container filled with liquid nitrogen.

e. **Heart:** The heart was placed in a storage bag, compressed, and frozen with a flat-bottomed metal container filled with liquid nitrogen.

f. **Bladder:** The bladder was flushed with isotonic saline and frozen in liquid nitrogen.

g. **Lymph node:** Approximately one-third of the mesenteric nodes were fixed in 10% neutral-buffered formalin; the remaining nodes were placed in a storage bag, compressed, and frozen with a flat-bottomed metal container filled with liquid nitrogen.

h. **Testes:** The testes were frozen separately in liquid nitrogen.

i. **Vas deferens/cauda epididymis:** The tissue was incised and placed in a petri dish with 1.5 mL of cold Dulbecco's phosphate-buffered saline. The suspended germ cells were filtered with a 40-μM cell strainer and a 1-mL portion was frozen in liquid nitrogen.

5. **Extraction of duodenal genomic DNA:** Lysis buffer (3 mL; containing RNase) was added to a Dounce-type homogenizer and cooled with ice, then a frozen tissue sample was added and homogenized with a pestle. The homogenate was added to a chilled tube containing 0.5 M sucrose (3 mL) and centrifuged. The supernatant was removed and cooled RNase-containing Dounce buffer (3 mL) was added and mixed well to yield a nuclear/cell suspension. Next, a proteinase K solution (3 mL) was added to the suspension and gently mixed. This suspension was incubated at 50°C for approximately three hours until it became clear. An equal volume (6 mL) of phenol/chloroform (1:1; v:v) was added, mixed, and centrifuged. The upper (aqueous) layer was collected and transferred into another tube and another equal volume of phenol/chloroform was added, mixed, and centrifuged; this procedure was repeated a third time. The water layer was then extracted with an equal volume of chloroform/isoamyl alcohol (24:1; v/v). The water layer was transferred into another tube and genomic DNA was extracted by gradually adding ethanol. Extracted genomic DNA was transferred into a microtube containing 70% ethanol and incubated for 10 minutes. The mixture was centrifuged, the supernatant was removed, and the remaining ethanol was allowed to evaporate. TE buffer (50 μL; not described) was added and the tube was incubated overnight at room temperature to dissolve the DNA residues. The DNA-containing solution was stored refrigerated after preparation.

6. **Preparation of test strains, packaging genomic DNA, and plating:** *Escherichia coli C* (*lacZ–*, *gal E–*) was cultured for packaging. Packaging was performed by using a commercially-available kit. One tube of packaging extract (red tube) was thawed and 10 μL of genomic DNA solution (adjusted to a concentration of about 200-600 μg/mL) was added. The tube was mixed and incubated at 30°C for 90 minutes. Next, a tube of packaging extract (blue tube) was thawed and 10 μL was transferred to the red tube and mixed. The mixture was incubated at 30°C for 90 minutes, diluted with 700 μL of buffer, and mixed yielding the packaged DNA sample.

   The *E. coli* suspensions for calculating total number of plaques (for titer) and for calculating mutant frequency (for selection) were dispensed into tubes. Then, the entire volume of the packaged DNA sample was added to the tube for selection and mixed. The tube was incubated at room temperature for about 30 minutes to allow the phage to infect the *E. coli*. A small quantity (30 μL) of the infected suspension was diluted 10-fold with broth

containing 10 mmol/L magnesium sulfate and 30 µL of the dilution was transferred to the tube for titering and stirred.

Magnesium sulfate solution (1M) was added to an agar solution at a volume ratio of 2:100 to make top agar for titration. A P-gal solution was added to the agar solution at a volume ratio of 2:100 to make the top agar for selection. Top agar was added to the tube for titering and mixed, and the contents were poured over a LB agar plate. Top agar was added to the tube for selection and the contents were poured over a LB agar plate in the same manner. The agar plates were incubated overnight at 37°C. In this single packaging, the number of plaques per animal were above 300,000; therefore, no further packaging was required.

7. **Plaque counting:** The number of plaques (N) in the plates for titration was counted and then the total number of plaques was calculated using the following equation:

$$\text{Total \# of plaques} = N \times \left(\frac{300\mu L}{30\ \mu L}\right) \times \left(\frac{2700\ \mu L}{30\ \mu L}\right)$$

$$= N \times 900$$

The number of plaques in the plates for selection was counted and recognized as mutant plaques.

The *lacZ* gene was selected as a reporter gene. The mutant frequency in a concerned organ was calculated by dividing the number of mutant plaques by the total number of plaques:

$$\text{Mutant frequency} = \frac{\text{\# of mutant plaques}}{\text{total \#of plaques}}$$

8. **Study validity:** The study was considered valid if the following conditions were met:

- The mutant frequency for the duodenum in the positive controls was markedly increased with a significant difference from the negative control; and
- The mutant frequency in the negative control should be within an acceptable range (mean ± 3 SD) relative to historical control data.

II. RESULTS

A. CLINICAL SIGNS: No treatment-related clinical signs of toxicity were observed.

B. BODY WEIGHTS AND BODY WEIGHT GAINS: Body weight and body weight gain data are presented in Table 2. In the 7000 ppm group, there were decreases in body weights ($p \leq 0.05$; ↓6%) on Day 15 and body weight gain during Days 1-31 (−0.1 g treated vs. 0.7 g control). In the positive control group, there was a decrease in body weight during Days 1-14 (↓8%).

TABLE 2. Mean (± SD) body weights and body weight gains (g) in transgenic mice treated with dicamba in the diet for up to 28 days. [a]

Day	Dose (ppm)				
	0	1200	3000	7000	ENU [b]
1	27.5 ± 1.4	27.4 ± 1.2	27.6 ± 1.2	27.4 ± 1.1	27.4 ± 1.0
3	27.2 ± 1.4	26.7 ± 1.3	27.0 ± 1.2	25.9 ± 1.2	---
8	26.4 ± 1.7	26.4 ± 1.1	27.0 ± 1.2	25.9 ± 1.0	---
14 [c]	---	---	---	---	25.2 ± 0.4
15	27.8 ± 1.6	27.1 ± 1.1	27.2 ± 1.5	26.0 ± 0.9* (↓6)	---
22	27.3 ± 1.9	27.0 ± 0.8	27.2 ± 1.7	25.8 ± 1.2	---
29	28.0 ± 2.0	27.4 ± 1.1	27.8 ± 1.9	26.5 ± 1.4	---
31	28.2 ± 2.1	27.7 ± 1.3	28.7 ± 2.0	27.3 ± 1.6	---
BWG Days 1-14	---	---	---	---	−2.2 ± 0.9
BWG Days 1-31	0.7 ± 1.7	0.3 ± 0.9	1.1 ± 1.1	−0.1 ± 1.0	---

a Data were obtained from Appendix 1 on pages 49-50 of MRID 51129105. N = 7.
b Positive control = 100 mg/kg/day *N*-ethyl-*N*-nitrosourea (ENU), administered by two i.p. injections on Days 3 and 4.
c ENU mice euthanized on Day 14.
--- Not applicable
* Significantly different from control; p≤0.05.

C. FOOD CONSUMPTION AND COMPOUND INTAKE: Food consumption data are presented in Table 3. In the 7000 ppm group, food consumption was decreased (p≤0.05) by 22% during Days 1-3.

Compound intake data are presented in Table 1.

TABLE 3. Mean (± SD) food consumption (g/mouse/day) in transgenic mice treated with dicamba in the diet for up to 28 days. [a]

Days	Dose (ppm)			
	0	1200	3000	7000
1-3	3.7 ± 0.6	3.9 ± 0.5	3.8 ± 0.4	2.9 ± 0.5* (↓22)
3-8	3.8 ± 0.6	3.9 ± 0.4	4.0 ± 0.5	3.2 ± 0.4
8-15	3.7 ± 0.4	3.5 ± 0.7	3.2 ± 0.7	3.1 ± 0.4
15-22	4.0 ± 0.3	4.2 ± 0.4	4.2 ± 0.6	4.0 ± 0.4
22-29	4.3 ± 0.5	4.3 ± 0.4	4.3 ± 0.6	4.0 ± 0.5

a Data were obtained from Appendix 3 on page 58 of MRID 51129105. N = 7.
* Significantly different from control; p≤0.05.

D. SACRIFICE AND PATHOLOGY

1. **Duodenum weights:** There were no effects of treatment on absolute or relative (to body) duodenum weights in the dicamba- or ENU-treated groups.

2. **Gross pathology:** There were no treatment-related effects noted at necropsy. The only macroscopic finding was a single, 4-mm white nodule in the right lung of a control mouse.

3. **Microscopic pathology:** No microscopic findings were reported.

E. MUTANT FREQUENCY: Mutant frequency data are reported in Table 4. Administration of dicamba in the diet did not increase mutant frequency. All mean values fell within the 95% confidence range (14.4×10^{-6} to 86.9×10^{-6}) calculated from the historical control data.

The positive control (ENU) produced a marked ($p \leq 0.05$) 10.6-fold increase in the mutant frequency.

TABLE 4. Mean (± SD) mutation data in the duodenum of transgenic mice treated with dicamba in the diet for up to 28 days. [a]

Parameter	Dose (ppm)				
	0	1200	3000	7000	ENU [b]
Plaque forming units	988,500 ± 286404.1	1,208,850 ± 365,388.7	1,054,050 ± 242,978.9	895,950 ±. 307,626.7	654,900 ± 159,210.7
# of mutants	75 ± 34.7	85 ± 48.0	81 ± 40.5	57 ± 12.6	520 ± 116.6
Mutant frequency (10^{-6})	75.3 ± 18.3	66.4 ± 18.4	75.3 ± 28.7	65.3 ± 9.9	799.2 ± 57.8*

a Data were obtained from Table 1 on page 47 of MRID 51129105. N = 6.
b Positive control = 100 mg/kg/day *N*-ethyl-*N*-nitrosourea (ENU), administered by two i.p. injections on Days 3 and 4.
* Significantly different from control; $p \leq 0.05$.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The negative control value was within the acceptable range of the historical control data and thus considered as valid. The mutant frequencies in the duodenum of the animals treated with Dicamba techn. (BAS 183 H; SAN837 techn.) did not show any increases as compared to the concurrent negative control value. All individual as well as group values were also within the historical control data.

The mutant frequencies in the duodenum in the positive control group, which was treated with *N*-ethyl-*N*-nitrosourea (ENU), increased and these increases were statistically significant compared with those of the negative control group. Therefore, the present study was judged to be properly conducted.

Considering all information available, including statistical analysis, it was concluded that Dicamba techn. (BAS 183 H; SAN837 techn.) did not induce gene mutations in the duodenum of transgenic mice (negative) under the conditions in this study.

B. REVIEWER COMMENTS: The Reviewers agree with the Investigators' conclusions.

There were no effects of treatment on clinical signs of toxicity, absolute or relative (to body) duodenum weights, or necropsy or microscopic findings. There were no changes in absolute or relative (to body) duodenum weights in the ENU-treated group.

In the 7000 ppm group, there were decreases in body weight ($p \leq 0.05$; ↓6%) on Day 15 and body weight gain during Days 1-31 (−0.1 g treated vs 0.7 g control). In the positive control group, there was a decrease in body weight during Days 1-14 (↓8%). Additionally, at 7000 ppm, food consumption was decreased ($p \leq 0.05$) by 22% during Days 1-3.

Administration of dicamba in the diet did not increase mutant frequency. All mean values fell within the 95% confidence range (14.4×10^{-6} to 86.9×10^{-6}) calculated from the historical control data. The positive control (ENU) produced a marked ($p \leq 0.05$) 10.6-fold increase in the mutant frequency.

This study is classified as **acceptable / non-guideline**.

C. STUDY DEFICIENCIES: The following deficiency was noted:

- The variance between nominal and actual dietary doses was greater than normally accepted (±15%).

Appendix 1. Dose range-finding study

In a non-guideline, dose range-finding study (MRID 51129106), groups of three male CD2F1/Slc (wild type for Muta™Mouse) mice/dose level were administered dicamba (89.8% a.i., batch # P.MG2726410) in the diet at dose levels of 0, 1000, 3000, or 10,000 ppm (equivalent to 0, 171, 434, and 1443 mg/kg/day) for 14 consecutive days. On Day 15, the mice were euthanized, necropsied, and the duodenum and liver were excised and weighed. The duodenum was examined microscopically.

All mice survived to scheduled euthanasia. There were no effects of treatment on clinical signs, body weights or body weight gains, food consumption, or gross or microscopic pathology.

At 10,000 ppm, absolute and relative (to body) liver weights were increased ($p<0.05$) by 32% and 29%, respectively, and absolute and relative duodenum weights were increased by 26% ($p<0.05$) and 28% (NS), respectively. Although there were no corroborating macroscopic or microscopic findings, the high dose for the main gene mutation assay study (MRID 51129105) was set at 7000 ppm and was expected to approximate the limit dose (1000 mg/kg/day) specified in OECD 488.

Appendix 2. Validation study

In an analytical method validation study (MRID 51129107), a method for the detection of dicamba (89.8% a.i.; batch P.MG2726410) in powdered CRF-1 basal diet (Oriental Yeast) was presented. Dicamba was incorporated into basal diet at two target concentrations of 1000 and 7000 ppm by grinding the test compound with a mortar and pestle and mixing an appropriate weight of the test compound with an appropriate weight of basal diet. The criteria for validation of the method included specificity, linearity of the calibration curve, accuracy, precision (repeatability), limit of quantification (LOQ) and limit of detection (LOD), and carry-over.

HPLC with a UV detection system was used to quantitate dicamba in the basal diet. Six matrix-matched calibration solutions were prepared from a stock solution of dicamba in acetonitrile (2.5 mg/100 mL, 5.0 mg/100 mL, 10.0 mg/100 mL, 15.0 mg/100 mL, 20.0 mg/100 mL, and 25.0 mg/100 mL).

The lowest calibration solution, solvent blank (acetonitrile), and basal diet extract were analyzed with a single injection. The method was considered specific if there was no response or a response <30% of the lowest calibration solution that corresponded to the retention time of dicamba in the calibration solution. The chromatogram of the calibration solution showed one peak (retention time [RT] approximately 3.5 minutes); chromatograms of the solvent blank and basal diet extract had no corresponding peaks. Therefore, the method was considered specific for dicamba.

The calibration solutions were analyzed with a single injection and the response (peak area) was correlated with the nominal concentrations by using regression analysis (without a weighting factor). The slope and intercept of the calibration curve, coefficient of determination (r^2), and back-calculated accuracies of the calibration solutions were calculated. The acceptance criteria were r^2 <0.990 and back-calculated accuracies of 100% ± 15%. The calibration curve was linear (slope = 2.821656; intercept = 0.032106), r^2 was >0.990 (r^2 = 0.999961), and the back-calculated accuracies all fell within the target range (−0.7% to 0.8%). Therefore, the calibration curve was accepted.

Accuracy and precision were determined in a single run. Five samples were taken from each of the dietary formulations (1000 and 7000 ppm) and extracted. Samples of the dietary formulations (5 g for the 7000 ppm formulation, 10 g for the 1000 ppm formulation) were placed in a 50-mL tube and 20 mL of 0.5 M sulfuric acid were added and shaken for 20 minutes. This mixture was extracted three times with 25 mL of acetonitrile for 30 minutes, the extracts were combined, made up to a final volume of 100 mL with acetonitrile, and filtered (0.2 µm). The extracts were analyzed with a single injection. Accuracy was calculated as:

$$\text{Accuracy} = \frac{\text{analyzed concentration}}{\text{target concentration}} \times 100$$

The relative standard deviation (%RSD) was calculated from the individual accuracies as:

$$\%\text{RSD} = \frac{\text{standard deviation}}{\text{mean accuracy}} \times 100$$

The acceptance criteria were that the mean accuracy fell within 70-110% of the nominal concentration (ideally 80-100%) and the %RSD for the formulations were ≤20% for precision. The mean accuracies were 99.9% and 104.7% of nominal and the %RSD were 4.5% and 1.9%, satisfying the accuracy and precision criteria.

For MRID 51129105, the lowest dietary concentration was 1000 ppm; therefore, the LOQ was defined as the lowest concentration of a sample where the accuracy and precision data fell within the tolerances. The LOQ was determined from the accuracy and precision data for the 1000 ppm formulation. The LOD was determined with a dicamba concentration of 2.0 mg/100 mL (20% of the LOQ or 200 ppm before extraction). A peak corresponding to the appropriate RT was clearly visible; therefore, the LOD was determined to be ≤200 ppm (from a 10-g sample).

Carry-over analysis was performed by injecting the highest calibration solution followed by two injections of basal diet extract. The acceptance criteria were to have no peaks corresponding to dicamba in the chromatograms or a peak <30% of the lowest calibration solution that corresponded to the retention time of dicamba in the calibration solution. There were no peaks corresponding to dicamba following injections of the basal diet extract; therefore, there was no carry-over from the injection of the highest calibration solution.

Stability analyses were not performed and were not considered necessary as a complete analytical run was performed in less than 24 hours.

It was concluded that the analytical method met the requirements for specificity, linearity, accuracy, repeatability, LOQ, and LOD for quantification of dicamba in powdered CRF-1 diet.